

Confirmation of Psoriasis Susceptibility Loci on Chromosome 6p21 and 20p13 in French Families

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Plaque psoriasis is a chronic inflammatory disorder of the skin. It is inherited as a multifactorial trait, with a strong genetic component. Linkage studies have identified a large number of disease loci, but very few could be replicated in independent family sets. In this study, we present the results of a genome-wide scan carried out in 14 French extended families. Candidate regions were then tested in a second set of 32 families. Analysis of the pooled samples confirmed linkage to chromosomes 6p21 (Z_{MLB} score = 3.5, $P = 0.0002$) and 20p13 (Z_{MLB} score = 2.9, $P = 0.002$), although there was little contribution of the second family set to the 20p13 linkage signal. Moreover, we identified four additional loci potentially linked to psoriasis. The major histocompatibility complex region on 6p21 is a major susceptibility locus, referred to as *PSORS1*, which has been found in most of the studies published to date. The 20p13 locus segregates independently of *PSORS1* in psoriasis families. It has previously been thought to be involved in the predisposition to psoriasis and other inflammatory disorders such as atopic dermatitis (AD) and asthma. Although psoriasis and AD rarely occur together, this reinforces the hypothesis that psoriasis is influenced by genes with general effects on inflammation and immunity.

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INTRODUCTION

Psoriasis is a chronic inflammatory dermatosis that affects 2–4% of the Caucasian population (Nevitt and Hutchinson, 1996). Clinically, the disorder is characterized by well-defined, inflammatory, indurated, scaly plaques generally occurring on extensor surfaces and in most cases on the scalp. Typically, the lesions wax and wane over the years. Based on the clinical aspects of the lesions, seven different subtypes have been defined (Krueger and Duvic, 1994). Plaque psoriasis is the most common type, accounting for more than 80% of all psoriasis cases. Although the prognosis is rarely fatal, psoriasis has a significant adverse effect on patients' quality of life (Krueger *et al.*, 2001). The impact for health care and cost are considerable (Elder *et al.*, 2001). Psoriasis is a multifactorial disease arising through a combination of both environmental and genetic risk factors,

which have been extensively documented (Bhalerao and Bowcock, 1998; Camp, 1998). Segregation analyses of families from large epidemiological studies show no clear pattern of inheritance (Pietrzyk *et al.*, 1982). The estimated sibling-recurrence risk ratio λ_s ranges from 4 to 11.5 (Hellgren, 1967; Farber *et al.*, 1974). Disease concordance rates are much higher in monozygotic twins (65–72%) than in dizygotic twins (15–30%), consistent with genetic factors playing a significant part in disease pathogenesis (Bhalerao and Bowcock, 1998).

All genome-wide studies have revealed highly significant linkage to a region on the major histocompatibility complex (MHC) at 6p21.3, referred to as *PSORS1* (psoriasis susceptibility 1, Nair *et al.*, 1997; Trembath *et al.*, 1997; Leder *et al.*, 1998; Samuelsson *et al.*, 1999; Enlund *et al.*, 1999a; Veal *et al.*, 2001; Zhang *et al.*, 2002; Sagoo *et al.*, 2004). Allele sharing between sib-pairs estimated the contribution to the familial clustering of disease (λ) to be $33 < \lambda < 50\%$ (Trembath *et al.*, 1997; The International Psoriasis Genetics Consortium, 2003). Therefore, although linkage to *PSORS1* is the strongest and most replicable, this locus does not explain the entire genetic predisposition and several genome-wide scans have been conducted in the search for additional psoriasis susceptibility loci. Seven additional susceptibility loci have been identified, namely *PSORS2* to *PSORS7* and *PSORS9*, respectively, on chromosomes 17q25 (Tomfohrde *et al.*, 1994), 4q34 (Matthews *et al.*, 1996; Nair *et al.*, 1997), 1q21 (Capon *et al.*, 1999), 3q21 (Enlund *et al.*, 1999b), 19p13 (Lee *et al.*, 2000), 1p32 (Veal *et al.*, 2001), and 4q31 (Zhang

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Abbreviations: *PSORS1*, psoriasis susceptibility 1; MLB method, maximum likelihood binomial method; MHC, major histocompatibility complex; HLA, human leukocyte antigen; NPL statistic, non parametric linkage statistic; SNP, single nucleotide polymorphism; AD, atopic dermatitis

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et al., 2002). Suggestive linkages have also been found on chromosomes 2q, 8q24, 20p13 (Nair *et al.*, 1997; Trembath *et al.*, 1997), 16q23 (Karason *et al.*, 2003), 3p21–23, 4q13, 5q31, 15q11 (Samuelsson *et al.*, 1999), 21q11–q21 (Lee *et al.*, 2000), 2p12–p14, 7, 14q22–q32 (Veal *et al.*, 2001), 9q33 (Zhang *et al.*, 2002), and 18p11 (Asumalahti *et al.*, 2003). More recently, a meta-analysis was conducted of the previous linkage scans for psoriasis susceptibility loci (Sagoo *et al.*, 2004). Apart from the major susceptibility locus *PSORS1*, only *PSORS2* (Tomfohrde *et al.*, 1994; Nair *et al.*, 1997; Samuelsson *et al.*, 1999; Zheng *et al.*, 2003), *PSORS3* (Matthews *et al.*, 1996; Nair *et al.*, 1997), *PSORS6* (Lee *et al.*, 2000; Veal *et al.*, 2001), *PSORS4* (Capon *et al.*, 2001), and the regions 3q21 (Samuelsson *et al.*, 1999), 5q31 (Friberg *et al.*, 2006), 16q12–q23 (Nair *et al.*, 1997; Karason *et al.*, 2003), and 20p13 (Nair *et al.*, 1997; Trembath *et al.*, 1997) have been detected in at least two studies.

Here we present the results of a linkage analysis performed on extended families collected in France. As a first step, a genome-wide scan with markers every 15 cM was carried out on 14 multigenerational kindreds. A higher density of microsatellite markers was used at some candidate loci reported in the literature. All markers suggesting linkage ($P < 0.01$) were then tested in a second set of 32 extended families. Our results confirm previous findings of linkage to *PSORS1* and support the presence of a susceptibility locus on chromosome 20p13, a region previously thought to be involved in the predisposition to psoriasis (Nair *et al.*, 1997; Trembath *et al.*, 1997) and other inflammatory disorders such as atopic dermatitis (AD) and asthma (Cookson *et al.*, 2001).

RESULTS

A genome-wide scan was conducted in a first set of 14 multigenerational psoriasis families (Table 1). Additional microsatellites were added to the 15 cM linkage panel at

some previously published candidate loci (Table S1). Multi-point linkage analysis using the maximum likelihood binomial (MLB) method allowed detection of linkage with $P \leq 0.001$, at *PSORS1* locus on 6p21 (maximum Z_{MLB} score = 3.6, $P = 0.0002$) and on chromosome 20p13 (maximum Z_{MLB} score = 3.1, $P = 0.001$). In addition, we found indication of linkage (with $P < 0.01$) to chromosomes 10q, 13q, 14q, and 16p (Table 2). To further characterize the linkage signal, a second set of 32 large families was genotyped at these six loci for a replication study. The structure of the families and the clinical characteristics of the patients of the two sets are summarized in Table 1 and are similar in the two groups. Furthermore, the distributions of affected sibships, presented in Table S2, do not differ in the two sets.

In set 2, the strongest indication of linkage was obtained at the *PSORS1* locus (Z_{MLB} score = 1.5, $P = 0.06$), but the suggested linkage to the 10q, 13q, 14q, and 20p regions in set 1 was not confirmed. However, analyses of the pooled samples confirmed linkage to both 6p21 and 20p13 (Z_{MLB} score = 3.5, $P = 0.0002$; and Z_{MLB} score = 2.9, $P = 0.002$, Table 2).

Second, we investigated the presence of interactions or genetic heterogeneity between regions retained as being potentially linked to psoriasis in the whole sample. We found no significant correlation between the max Z_{MLB} scores obtained in the six detected regions (6p21, 10q, 13q, 14q, 16p, and 20p13), when these were analyzed two by two. Similarly, the conditional MLB analyses did not reveal any significant interaction or heterogeneity between markers of the detected regions, including the 6p21 (data not shown).

DISCUSSION

In this study, the regions 6p21, 10q, 13q, 14q, 16p, and 20p13 were detected as susceptibility loci in a first set of 14

Table 1. Clinical characteristics of the 46 psoriasis families

	Set 1	Set 2	Total
Number of families	14	32	46
Number of affected individuals	163	205	368
Male/female ratio of affected	1.01	0.93	0.97
Total family members (genotyped)	556 (466)	618 (540)	1174 (1006)
Consanguineous family	—	1	1
Two-generation families	—	7	7
Three-generation families	10	23	33
Four-generation families	4	1	5
Mean number of affected by family	11.6	6.4	8.0
Mean/median age of onset	19.9/14.0	19.3/12.7	19.5/13.3
Mean/median age at examination	41.9/18.5	43.3/17.6	42.7/18.0
Patients with plaque psoriasis (%)	97	95	96
Confirmation of the diagnosis by an independent physician (%)	79.9	70.2	74.7

Table 2. Multipoint linkage analysis (performed with MLB and MERLIN programs)

Region	Marker	Distance from pter. (cM)	Z _{MLB} score (P-value)			Z _{MERLIN} score (P-value)
			Set 1	Set 2	Set 1+Set 2	Set 1+Set 2
6p	D6S1542	47.7	3.4 (0.0003)	1.5 (–)	3.5 (0.0002)	1.31 (–)
	D6S1568	47.7	3.4 (0.0003)	1.5 (–)	3.5 (0.0002)	1.49 (–)
	TNF α	47.7	2.9 (0.002)	1.3 (–)	3.0 (0.001)	1.31 (–)
	D6S1560	47.7	2.9 (0.002)	1.1 (–)	2.8 (0.003)	1.31 (–)
	D6S1618	47.7	3.1 (0.001)	1.0 (–)	3.0 (0.003)	1.31 (–)
	D6S439	48.2	3.6 (0.0002)	0.8 (–)	3.2 (0.0007)	1.24 (–)
10q	D10S603	124	0.7 (–)	1.1 (–)	1.3 (–)	1.70 (0.04)
	D10S1731	134	2.3 (0.01)	0.4 (–)	1.9 (0.03)	2.20 (0.01)
	D10S587	148	0.3 (–)	0.3 (–)	0.4 (–)	0.65 (–)
13q	D13S154	75	1.2 (–)	0.6 (–)	1.3 (–)	0.37 (–)
	D13S280	85	2.9 (0.002)	0.5 (–)	2.4 (0.008)	1.65 (0.05)
	D13S286	94	1.2 (–)	1.2 (–)	1.7 (0.04)	1.10 (–)
14q	D14S63	69	1.6 (0.05)	0.0 (–)	1.1 (–)	1.06 (–)
	D14S1028	81	2.3 (0.01)	0.0 (–)	1.6 (0.05)	1.20 (–)
	D14S1052	94	1.7 (0.04)	0.0 (–)	0.5 (–)	–0.02 (–)
16p	D16S423	10	1.0 (–)	0.0 (–)	0.2 (–)	0.14 (–)
	D16S3075	23	2.4 (0.008)	0.8 (–)	2.3 (0.01)	0.67 (–)
	D16S3103	32	1.7 (0.04)	0.6 (–)	1.6 (0.05)	0.92 (–)
20p	D20S864	0	3.1 (0.001)	0.9 (–)	2.9 (0.002)	1.62 (0.05)
	D20S116	11	2.8 (0.003)	0.6 (–)	2.4 (0.008)	1.48 (–)

(–): $P > 0.05$.

psoriasis families. Despite the absence or weak replication in a second set of 32 families, the results in the whole sample set for the 6p21 and 20p13 regions, which showed the highest Z_{MLB} scores, remained unchanged when compared with those in the first set of data. The fact that the second set of families does not provide evidence for linkage when analyzed on its own may be because of a false detection of linkage in the first set owing to multiple testing or to heterogeneity between samples, which however was non-significant when tested in the predivided sample set (data not shown). Note that this test, as all tests of heterogeneity, is not very powerful. The lack of replication may be also due to the complexity of the disease depending on numerous genes with major genetic heterogeneity; different genes would then be detected from one study to another. Finally, it may simply be because of a lack of power of replication due to the variability of the statistic as has been shown for the Maximum Lod Score statistic (Clerget-Darpoux *et al.*, 2001).

Those loci that have been identified as potentially involved in psoriasis susceptibility in only one or two studies but are not picked up in other studies may be false positives, be peculiar to the population used, or other studies may just lack the power to detect them. It has also been shown that some of these loci are linked to a particular subphenotype of psoriasis. For example, the 16q locus was recently shown to

be a “psoriasis arthritis” locus in the Iceland study (Karason *et al.*, 2005). In this study, more than 95% of patients suffer from plaque psoriasis, and there was no evidence of linkage at the 16q locus.

Another possible reason for the lack of replication of some results could be the large effect of the *PSORS1* locus masking the other loci involved in psoriasis. Because of this potentially strong effect, it has been suggested that studies should condition on the effect of the *PSORS1* locus. This was carried out here by using the conditional MLB method, which, however, did not lead to significant increase of linkage score when conditioning on *PSORS1* in any regions detected by the previous linkage analyses. Moreover, neither interaction nor heterogeneity was shown here between any of these regions.

For linkage analysis the pedigrees were broken into nuclear families because of facility of computing, and we chose the MLB method because it uses simultaneous information on the entire set of affected sibships. Moreover, for complex diseases depending on numerous genes and using an outbred population, it may be more appropriate to use small familial structures such as nuclear families rather than large pedigrees (Abel *et al.*, 1998). When analyzing whole pedigrees, the power to detect linkage may be decreased by genetic heterogeneity within pedigrees. However, for the regions 6p and 20p, for which linkage to

psoriasis could be confirmed by using the MLB method on pedigrees separated into nuclear families, we also applied the program SIMWALK (Sobel and Lange, 1996) using the non parametric linkage (NPL) statistic on the whole pedigrees, because this program makes it possible to analyze pedigrees as large as those present in our sample. With this analysis, there was weak indication of linkage to the 6p region and there was no indication of linkage to the 20p region (data not shown).

In addition, we also performed NPL analyses using the affected sib-pairs (ASP) method implemented in MERLIN (Abecasis *et al.*, 2002). Families were too large to perform multipoint linkage analyses on the whole pedigrees using this program, and they too had to be cut into nuclear families. No significant linkage was detected with this method on any chromosome. Results obtained with MERLIN for the regions 6p21, 10q, 13q, 14q, 16p, and 20p13 are presented in Table 2. Apart from locus on chromosome 10q, the NPL statistic generated by MERLIN suggested weaker linkage signals than the Z_{MLB} scores. The power to detect linkage depends on the informativity of the data including the familial structures of the pedigrees. In particular, for the MLB method, this informativity depends mostly on the number and size of the affected sibships present in the sample. Thus, the loss of power with MERLIN may be attributable to the fact that this program cannot use simultaneous information on the entire set of affected sibships as does the MLB method. Therefore, the MLB method seems more appropriate here, as there were extended affected sibships in the pedigrees included in this study.

The number of genes involved in the pathogenesis of psoriasis and their chromosomal location are presently unknown and only the MHC region (*PSORS1* locus) has met the accepted criteria for genome-wide significance and has been consistently replicated in independent studies (Elder *et al.*, 2001). Refinement of the interval using single-nucleotide polymorphisms (SNPs) has been carried out by several teams, and associations between psoriasis and SNPs have been reported without clear identification of a gene in which mutations or polymorphisms could explain the disorders, although some alleles in the MHC region are good candidates, particularly human leukocyte antigen (HLA)-Cw0602 (Jenisch *et al.*, 1998; Balendran *et al.*, 1999; Oka *et al.*, 1999; Nair *et al.*, 2000; Helms *et al.*, 2005; Holm *et al.*, 2005). Association between HCR*WWCC (Asumalahti *et al.*, 2000, 2002) and CDSN*5 (Ishihara *et al.*, 1996; Allen *et al.*, 1999; Jenisch *et al.*, 1999; Capon *et al.*, 2004; Lench *et al.*, 2005; Chang *et al.*, 2006) in strong linkage disequilibrium with (HLA)-Cw0602, and psoriasis has also been demonstrated. We also confirmed the associations with these three tightly linked susceptibility alleles and psoriasis in our family set (data not shown). Based on the linkage and association studies, the *PSORS1* locus seems to explain 30–50% of psoriasis susceptibility. Other minor susceptibility loci are thus likely to exist. The detection of the minor loci has, however, proved to be difficult because of locus heterogeneity, which makes the replication of the linkage difficult in different ethnic groups (Capon *et al.*, 2002).

The 10q region has been reported previously in a meta-analysis performed on six psoriasis genome-wide scans (Sagoo *et al.*, 2004), but in our study Z_{MLB} score did not reach a significant value when analyzing the whole sample. Although showing weaker linkage in this study, the 13q and 14q regions have been reported to be linked to psoriasis (Veal *et al.*, 2001; Sagoo *et al.*, 2004). However, for replication studies of published regions, thresholds for the detection of linkage may be less stringent. The 16p region, which has not been reported elsewhere, is of borderline significance and could be a false positive result or be peculiar to the French population used. To conclude, the most significant non-MHC locus in French patients was on chromosome 20p. Our finding is reinforced by the implication of this region in two other independent genome-wide studies. The first one was carried out on 41 multiplex European families (Trembath *et al.*, 1997) and the second on 115 American and German families (Nair *et al.*, 1997).

A genetic component to autoimmune susceptibility has been clearly shown by twin and adoption studies and by increased risk to siblings (Vyse and Todd, 1996). The 20p locus was not found initially among the non-MHC loci that could collectively contribute to disease susceptibility in human autoimmune diseases (Becker *et al.*, 1998), but it is also of note that a linkage to this locus has been found afterwards in a genome screen for childhood AD (Cookson *et al.*, 2001). Although immunologic processes of psoriasis and AD are quite different (psoriasis is TH-1 mediated and AD is a TH-2 reaction), both diseases are characterized by dry, scaly skin, disturbed epidermal differentiation, and an inflammation that is responsive to T-cell-specific agents. The two diseases rarely occur together in the same patient. However, linkage of both diseases to the 20p13 locus indicates that psoriasis and AD are influenced by genes with general effects on cutaneous inflammation and immunity.

The 17Mb interval on chromosome 20 contains 428 known genes. A refinement of this interval is obviously needed before any relationship between psoriasis and a particular gene in this region could be considered, even though the β -defensin genes, known to be implicated in immunologic defense against bacteria, fungi, and some enveloped viruses (Ganz 2002; Yang *et al.*, 2002) and *TGM3* encoding a transglutaminase, for which the transcript is upregulated in psoriatic skin *versus* normal skin (Bowcock *et al.*, 2001), are good candidates. But there are undoubtedly additional compelling candidates. Replication of our findings is necessary and linkage disequilibrium studies using SNPs across this interval for positional cloning may ultimately identify the causative gene.

MATERIALS AND METHODS

Families

Families with psoriasis were recruited through a media campaign between 1996 and 2001 at G  n  thon, using posters in the Paris M  tro and information in news magazines, radio, and television (Mah   *et al.*, 2002). From 50,000 phone calls received by a toll-free telephone number, 108 families with at least eight putative affected members were identified, most of them with an apparent autosomal

dominant mode of inheritance of psoriasis. Clinical diagnoses were checked by systematic telephone calls to every family member, affected or non-affected, for each family, at least twice during 4 years by dermatologists using a standard questionnaire available on request. The attending physician of each patient was also contacted, mainly by mail, which led to confirmation of the diagnosis in over 75% of cases. Material was available for 46 families from France. They were split into two sets to reduce the genotyping effort. The structure of the 46 families and the clinical characteristics of the patients are summarized in Table 1. This study was approved by the Ethics Committee of Le Kremlin-Bicêtre Hospital in 1995 (CCPPRB). All subjects participating in this study provided informed consent. The study was conducted in concordance with the Declaration of Helsinki Principles.

Genotyping

DNA was extracted from whole blood using the standard phenol/chloroform procedure. A genome-wide scan using 260 polymorphic microsatellite markers from Généthon (Evry, France) was carried out as described previously (Dib *et al.*, 1996). The marker map position was based on the Marshfield map (<http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp>). Markers were spaced at an average distance of 15 cM. When suggestive linkage was found in the first set of 14 families, the locus was analyzed in the second set using 2–6 adjacent microsatellite markers with the exception of the 6p21 region (13 markers) and the 8q24 region (nine markers). The characteristics of these markers are described in Table S1.

Linkage analysis

Because of their large size, the complete pedigrees could not be analyzed by the computer programs currently used for linkage analysis, such as GENEHUNTER (Kruglyak *et al.*, 1996), Allegro (Gudbjartsson *et al.*, 2000), or MERLIN (Abecasis *et al.*, 2002), which have shown to be comparable (Abecasis *et al.*, 2002; Dudbridge, 2003). Other programs such as LINKAGE (Lathrop *et al.*, 1984) and SIMWALK (Sobel and Lange, 1996) could be used, but only for a limited number of markers or using a very long computing time.

When using the MLB method (Majumder and Pal, 1987; Satsangi *et al.*, 1996, Abel and Muller-Myhsok, 1998) or MERLIN, the pedigrees were therefore broken into nuclear families using the Mega2 program (Mukhopadhyay *et al.*, 2005). The first set of 14 pedigrees and the second set of 32 pedigrees produced 32 and 45 nuclear families, respectively, each with at least two affected sibs, which were used for linkage analyses. The distribution of sibships according to the number of affected sibs for each set of the 46 pedigrees is shown in Table S2.

Multipoint NPL analyses were performed on the ASP using MERLIN (Abecasis *et al.*, 2002). The ASP method calculates inheritance distribution for sets of affected pairs and then uses a score function to determine significance of linkage. We used the NPL_{All} statistic, which estimates identical-by-descent allele sharing among all affected members and is averaged over all possible inheritance patterns, normalized, and weighted across pedigrees. Under the null hypothesis of no linkage, the NPL statistic is distributed asymptotically as a standard normal random variable. Results are reported in terms of an NPL Z score (Z_{MERLIN}) and its associated one-sided P-value.

Because most of the pedigrees included in this study were complicated and their information may not be fully utilized by the NPL statistics, we applied another multipoint analysis using the MLB model-free method (Majumder and Pal, 1987; Satsangi *et al.*, 1996; Abel and Muller-Myhsok, 1998); this method does not require specification of the underlying genetic model for the trait investigated, and has the additional advantage of being applicable to complete sibships of affected individuals. The principle of the method is based on the binomial distribution of the number of affected sibs receiving a given parental allele. The likelihood contribution for meioses from a heterozygous parent with n affected offspring of which m inherited one marker allele and $n-m$ the other is equal to $[\alpha^\mu(1-\alpha)^{n-\mu} + \alpha^{n-\mu}(1-\alpha)^\mu]$. For the whole family, the contribution is the product of the two parental contributions. The product of the likelihoods over all families is maximised over α , with α being the probability for an affected sib to receive the marker allele transmitted with the disease allele. The test for linkage is performed using a likelihood ratio test statistic, $\Lambda = 2 \ln[L(\alpha)/L(\alpha = 0.5)]$, with α being equal to 0.5 under the null hypothesis of no linkage and $\alpha > 0.5$ under the hypothesis of linkage. The statistic Λ is distributed asymptotically as a mixture distribution of $0.5 \chi^2_{0df}$ and $0.5 \chi^2_{1df}$, and $Z_{MLB} = \Lambda^{0.5}$ is a one-sided standard normal deviate. All these analyses were conducted using the multipoint approach with the program MLBGH (Abel *et al.*, 1998). Allelic frequencies of the markers were estimated from the founders and unrelated individuals from pedigrees.

Results from the different data sets and for the whole sample were not corrected for multiple testing. It is known that correction for multiple testing of a genome scan is generally difficult to apply. A simple correction of Bonferroni type according to the total number of analyzed markers is conservative, because of linkage between the markers, particularly when the mapping was very fine in some regions, which is the case in our study. The linkage thresholds as proposed by Lander and Kruglyak (1995) are also conservative, as they have been computed under the assumption of a complete dense genome map.

Two loci and heterogeneity model analysis

Correlations between Z_{MLB} scores of loci with the maximum scores in the regions detected by previous linkage analyses were computed. In the case of significant correlation, linkage analysis with the MLB method at one of the loci was performed conditionally to the other locus, following a similar approach to the one developed by Cox *et al.* (1999) using the NPL score. Conditional linkage analysis was performed by setting a weight of 1 to the families with a Z_{MLB} score > 0 at the second locus and a weight of 0 to families with a Z_{MLB} score $= 0$ in the case of positive correlation (interaction model). Conversely, a weight of 0 was assigned to families with a Z_{MLB} score > 0 and a weight of 1 to the other families in the case of negative correlation (heterogeneity model). Absence of interaction or of heterogeneity is tested by measuring the significance of the increase of the MLB score using the non-conditional *versus* the conditional approach, the difference between the $(Z_{MLB})^2$ following asymptotically a χ^2 test with 1 df (Figure 1).

Electronic database information

Online Mendelian Inheritance in Man: <http://www.ncbi.nlm.nih.gov/Omim/searchomim.html> for psoriasis susceptibility 1 (PSORS1,

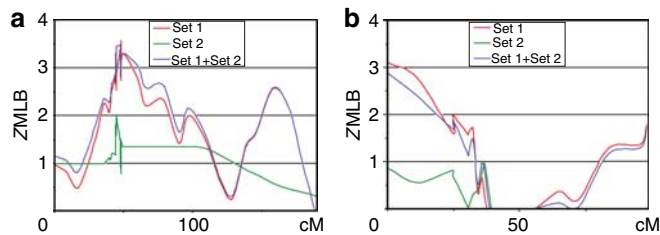


Figure 1. Graphical representation of the linkage signal for the two most significant regions (MLB method). (a) Result for chromosome 6. (b) Result for chromosome 20.

OMIM 177900); psoriasis susceptibility 2 (PSORS2, OMIM 602723); psoriasis susceptibility 3 (PSORS3, OMIM 601454); psoriasis susceptibility 4 (PSORS4, OMIM 603935); psoriasis susceptibility 5 (PSORS5, OMIM 604316); psoriasis susceptibility 6 (PSORS6, OMIM 605364); psoriasis susceptibility 7 (PSORS7, OMIM 605606); psoriasis susceptibility 9 (PSORS9, OMIM 607857).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Additional microsatellites genotyped at candidate loci in Set1 or in set1 + set2 (6p, 10q, 13q, 14q, 16p, and 20p).

Table S2. Distribution of families according to the number of affected sibs used for linkage analyses (i.e. families with at least two affected sibs).

REFERENCES

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin – rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 30:97–101
- Abel L, Muller-Myhsok B (1998) Robustness and power of the maximum-likelihood-binomial and maximum-likelihood-score methods, in multi-point linkage analysis of affected-sibship data. *Am J Hum Genet* 63:638–47
- Abel L, Alcais A, Mallet A (1998) Comparison of four sib-pair linkage methods for analyzing sibships with more than two affecteds: interest of the binomial maximum likelihood approach. *Genet Epidemiol* 15:371–90
- Allen MH, Veal C, Faassen A, Powis SH, Vaughan RW, Trembath RC *et al.* (1999) A non-HLA gene within the MHC in psoriasis. *Lancet* 353:1589–90
- Asumalahti K, Laitinen T, Itkonen-Vatjus R, Lokki ML, Suomela S, Snellman E *et al.* (2000) A candidate gene for psoriasis near HLA-C, HCR (Pg8), is highly polymorphic with a disease-associated susceptibility allele. *Hum Mol Genet* 9:1533–42
- Asumalahti K, Laitinen T, Lahermo P, Suomela S, Itkonen-Vatjus R, Jansen C *et al.* (2003) Psoriasis susceptibility locus on 18p revealed by genome scan in Finnish families not associated with PSORS1. *J Invest Dermatol* 121:735–40
- Asumalahti K, Veal C, Laitinen T, Suomela S, Allen M, Elomaa O *et al.* (2002) Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus. *Hum Mol Genet* 11:589–97
- Balendran N, Clough RL, Arguello JR, Barber R, Veal C, Jones AB *et al.* (1999) Characterization of the major susceptibility region for psoriasis at chromosome 6p21.3. *J Invest Dermatol* 113:322–8
- Becker KG, Simon RM, Bailey-Wilson JE, Freidlin B, Biddison WE, McFarland HF *et al.* (1998) Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. *Proc Natl Acad Sci USA* 95:9979–84
- Bhalerao J, Bowcock AM (1998) The genetics of psoriasis: a complex disorder of the skin and immune system. *Hum Mol Genet* 7:1537–45
- Bowcock AM, Shannon W, Du F, Duncan J, Cao K, Aftergut K *et al.* (2001) Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* 10:1793–805
- Camp RDR (1998) Rook/Wilkinson/Ebling. in: (Champion RH, Burton JL, Burns DA, Breathnach SM, eds), 6th ed *Textbook of dermatology*, 1589–649. Oxford: Blackwell Science
- Capon F, Allen MH, Ameen M, Burden AD, Tillman D, Barker JN *et al.* (2004) A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. *Hum Mol Genet* 13:2361–8
- Capon F, Munro M, Barker J, Trembath R (2002) Searching for the major histocompatibility complex psoriasis susceptibility gene. *J Invest Dermatol* 118:745–51
- Capon F, Semprini S, Chimenti S, Fabrizi G, Zambruno G, Murgia S *et al.* (2001) Fine mapping of the PSORS4 psoriasis susceptibility region on chromosome 1q21. *J Invest Dermatol* 116:728–30
- Capon F, Semprini S, Dallapiccola B, Novelli G (1999) Evidence for interaction between psoriasis-susceptibility loci on chromosomes 6p21 and 1q21. *Am J Hum Genet* 65:1798–800
- Chang YT, Chou CT, Shiao YM, Lin MW, Yu CW, Chen CC *et al.* (2006) Psoriasis vulgaris in Chinese individuals is associated with PSORS1C3 and CDSN genes. *Br J Dermatol* 155:663–9
- Clerget-Darpoux F, Selinger-Leneman H, Babron MC (2001) Why do complex traits resist DNA analysis? *Inter J Hum Genet* 1:55–63
- Cookson WO, Ubhi B, Lawrence R, Abecasis GR, Walley AJ, Cox HE *et al.* (2001) Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nat Genet* 27:372–3
- Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI *et al.* (1999) Loci on chromosome 2 (NIDDM1) and 15 interact to increase susceptibility diabetes in Mexican Americans. *Nat Genet* 21:213–5
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A *et al.* (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–4
- Dudbridge F (2003) A survey of current software for linkage analysis. *Hum Genomics* 1:63–5
- Elder JT, Nair RP, Henseler T, Jenisch S, Stuart P, Chia N *et al.* (2001) The genetics of psoriasis 2001: the odyssey continues. *Arch Dermatol* 137:1447–54
- Enlund F, Samuelsson L, Enerback C, Inerot A, Wahlstrom J, Yhr M *et al.* (1999a) Analysis of three suggested psoriasis susceptibility loci in a large Swedish set of families: confirmation of linkage to chromosome 6p (HLA region), and to 17q, but not to 4q. *Hum Hered* 49:2–8
- Enlund F, Samuelsson L, Enerback C, Inerot A, Wahlstrom J, Yhr M *et al.* (1999b) Psoriasis susceptibility locus on chromosome 3q21 identified in patients from southwest Sweden. *Eur J Hum Genet* 7:783–90
- Farber EM, Nall ML, Watson W (1974) Natural history of psoriasis in 61 twin pairs. *Arch Dermatol* 109:207–11
- Friberg C, Björck K, Nilsson S, Inerot A, Wahlstrom J, Samuelsson L (2006) Analysis of chromosome 5q31–32 and psoriasis: confirmation of a susceptibility locus but no association with SNPs within SLC22A4 and SLC22A5. *J Invest Dermatol* 126:998–1002
- Ganz T (2002) Immunology. Versatile defensins. *Science* 298:977–9
- Gudbjartsson DF, Jonasson K, Frigge ML, Kong A (2000) Allegro, a new computer program for multipoint linkage analysis. *Nat Genet* 25:12–3
- Hellgren L (1967) *Psoriasis*. Almqvist & Wiksell: Stockholm
- Helms C, Saccone NL, Cao L, Daw JA, Cao K, Hsu TM *et al.* (2005) Localization of PSORS1 to a haplotype block harboring HLA-C and distinct from corneodesmosin and HCR. *Hum Genet* 118:466–76

- Holm SJ, Sanchez F, Carlen LM, Mallbris L, Stahle M, O'Brien KP (2005) HLA-Cw*0602 associates more strongly to psoriasis in the Swedish population than variants of the novel 6p21.3 gene PSORS1C3. *Acta Derm Venereol* 85:2-8
- Ishihara M, Yamagata N, Ohno S, Naruse T, Ando A, Kawata H et al. (1996) Genetic polymorphisms in the keratin-like S gene within the human major histocompatibility complex and association analysis on the susceptibility to psoriasis vulgaris. *Tissue Antigens* 48:182-6
- Jenisch S, Henseler T, Nair RP, Guo SW, Westphal E, Stuart P et al. (1998) Linkage analysis of human leukocyte antigen (HLA) markers in familial psoriasis: strong disequilibrium effects provide evidence for a major determinant in the HLA-B/C region. *Am J Hum Genet* 63:191-9
- Jenisch S, Koch S, Henseler T, Nair RP, Elder JT, Watts CE et al. (1999) Corneodesmosin gene polymorphism demonstrates strong linkage disequilibrium with HLA and association with psoriasis vulgaris. *Tissue Antigens* 54:439-49
- Karason A, Gudjonsson JE, Jonsson HH, Hauksson VB, Runarsdottir EH, Stefansson K et al. (2005) Genetics of psoriasis in Iceland: evidence for linkage of subphenotypes to distinct loci. *J Invest Dermatol* 124:1177-85
- Karason A, Gudjonsson JE, Upmanyu R, Antonsdottir AA, Hauksson VB, Runarsdottir EH et al. (2003) A susceptibility gene for psoriatic arthritis maps to chromosome 16q: evidence for imprinting. *Am J Hum Genet* 72:125-31
- Krueger G, Koo J, Lebwohl M, Menter A, Stern RS, Rolstad T (2001) The impact of psoriasis on quality of life: results of a 1998 National Psoriasis Foundation patient-membership survey. *Arch Dermatol* 137:280-4
- Krueger GG, Duvic M (1994) Epidemiology of psoriasis. *J Invest Dermatol* 102:14S-8S
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347-63
- Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241-7
- Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443-6
- Leder RO, Mansbridge JN, Hallmayer J, Hodge SE (1998) Familial psoriasis and HLA-B: unambiguous support for linkage in 97 published families. *Hum Hered* 48:198-211
- Lee YA, Ruschendorf F, Windemuth C, Schmitt-Egenolf M, Stadelmann A, Nurnberg G et al. (2000) Genomewide scan in German families reveals evidence for a novel psoriasis-susceptibility locus on chromosome 19p13. *Am J Hum Genet* 67:1020-4
- Lench N, Iles MM, Mackay I, Patel R, Sagoo GS, Ward SJ (2005) Single-point haplotype scores telomeric to human leukocyte antigen-C give a high susceptibility major histocompatibility complex haplotype for psoriasis in a caucasian population. *J Invest Dermatol* 124:545-52
- Mahé E, Lahfa M, Mansouri S, Mosharraf-Olmolk H, Le Rebours J, Prud'homme JF et al. (2002) Physicians' response to a letter to confirm diagnosis in a genetic study of psoriasis. *Eur J Dermatol* 12:66-9
- Majumder PP, Pal N (1987) Nonrandom segregation: uniformly most powerful test and related considerations. *Genet Epidemiol* 4:277-87
- Matthews D, Fry L, Powles A, Weber J, McCarthy M, Fisher E et al. (1996) Evidence that a locus for familial psoriasis maps to chromosome 4q. *Nat Genet* 14:231-3
- Mukhopadhyay N, Almasly L, Schroeder M, Mulvihill WP, Weeks DE (2005) Mega2: data-handling for facilitating genetic linkage and association analyses. *Bioinformatics* 21:2556-7
- Nair RP, Henseler T, Jenisch S, Stuart P, Bichakjian CK, Lenk W et al. (1997) Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. *Hum Mol Genet* 6:1349-56
- Nair RP, Stuart P, Henseler T, Jenisch S, Chia NV, Westphal E et al. (2000) Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C. *Am J Hum Genet* 66:1833-44
- Nevitt GJ, Hutchinson PE (1996) Psoriasis in the community: prevalence, severity and patients' beliefs and attitudes towards the disease. *Br J Dermatol* 135:533-7
- Oka A, Tamiya G, Tomizawa M, Ota M, Katsuyama Y, Makino S et al. (1999) Association analysis using refined microsatellite markers localizes a susceptibility locus for psoriasis vulgaris within a 111 kb segment telomeric to the HLA-C gene. *Hum Mol Genet* 8:2165-70
- Pietrzyk JJ, Turowski G, Kapinska-Mrowka M, Rozanski B (1982) Family studies in psoriasis. I. Complex segregation analysis. *Arch Dermatol Res* 273:287-94
- Sagoo GS, Tazi-Ahnini R, Barker JW, Elder JT, Nair RP, Samuelsson L et al. (2004) Meta-analysis of genome-wide studies of psoriasis susceptibility reveals linkage to chromosomes 6p21 and 4q28-q31 in Caucasian and Chinese Hans population. *J Invest Dermatol* 122:1401-5
- Samuelsson L, Enlund F, Torinsson A, Yhr M, Inerot A, Enerback C et al. (1999) A genome-wide search for genes predisposing to familial psoriasis by using a stratification approach. *Hum Genet* 105:523-9
- Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K et al. (1996) Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 14:199-202
- Sobel E, Lange K (1996) Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker sharing statistics. *Am J Hum Genet* 58:1323-37
- The International Psoriasis Genetics Study (2003) Assessing linkage to 14 candidate susceptibility loci in a cohort of 942 affected sib pairs. *Am J Hum Genet* 73:430-7
- Tomfohrde J, Silverman A, Barnes R, Fernandez-Vina MA, Young M, Lory D et al. (1994) Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. *Science* 264:1141-5
- Trembath RC, Clough RL, Rosbotham JL, Jones AB, Camp RD, Frodsham A et al. (1997) Identification of a major susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis. *Hum Mol Genet* 6:813-20
- Veal CD, Clough RL, Barber RC, Mason S, Tillman D, Ferry B et al. (2001) Identification of a novel psoriasis susceptibility locus at 1p and evidence of epistasis between PSORS1 and candidate loci. *J Med Genet* 38:7-13
- Vyse TJ, Todd JA (1996) Genetic analysis of autoimmune disease. *Cell* 85:311-8
- Yang D, Biragyn A, Kwak LW, Oppenheim JJ (2002) Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol* 23:291-6
- Zhang XJ, He PP, Wang ZX, Zhang J, Li YB, Wang HY et al. (2002) Evidence for a major psoriasis susceptibility locus at 6p21(PSORS1) and a novel candidate region at 4q31 by genome-wide scan in Chinese Hans. *J Invest Dermatol* 119:1361-6
- Zheng J, Jin S, Shi R (2003) Confirmation of PSORS psoriasis susceptibility loci in a Chinese population. *Arch Dermatol Res* 295:14-8